Melanocortin Receptors 1 and 5 Might Mediate Inhibitory Effects of α-Melanocyte-Stimulating Hormone on Antigen-Induced Chronic Allergic Skin Inflammation in IgE Transgenic Mice

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TO THE EDITOR

Atopic dermatitis (AD) is a chronic allergic disease in skin with severe irritation and inflammation, and most patients with AD show elevated plasma levels of IgE against large numbers of allergens. It is known that IgE is involved in type I allergy, which is an immediate hypersensitivity through mast cells with IgE. However, it is not clear how IgE affects chronic allergic diseases such as AD. Anti-IgE antibody (omalizumab) has been shown to improve symptoms in not only allergic rhinitis, which is a typical type I allergy disease, but also AD and chronic asthma, which are chronic allergic diseases (Sheinkopf *et al.*, 2008). We focused on elucidating the mechanism for IgE-mediated chronic inflammation to find and establish a original treatment for chronic allergic diseases.

In 2,4,6-trinitrophenol-specific IgE transgenic mice (TNP-IgE Tg mice), a single subcutaneous injection of a multivalent antigen such as TNP-conjugated ovalbumin (TNP_{11}-OVA) to the ear induces tri-phase ear swelling (Matsuoka *et al.*, 1999; Sato *et al.*, 2003). The immediate-phase (within 1 hour after antigen challenge) and late-phase (within 6–10 hours after antigen challenge) ear swelling are typical responses of type I allergy, whereas the third-phase ear swelling (began 2 days later and peaked on days 3–4 after antigen challenge) especially reflects chronic IgE-mediated severe inflammation, which is induced by the multivalent antigen (Sato *et al.*, 2003). In the histological change of the third-phase ear swelling, massive infiltration of inflammatory cells such as eosinophils, neutrophils, and hyperplastic epidermis with hyperkeratosis were observed. Anti-histamine drugs did not show any significant effect on the third-phase swelling, whereas Cyclosporine A inhibited swelling and cellular infiltration (Sato *et al.*, 2003). Moreover, we showed that a topical steroid strongly inhibited swelling and cellular infiltration (Supplementary Figure S1 online). The third-phase ear swelling in TNP-IgE Tg mice has hallmarks of chronic allergic inflammation in AD at both points of IgE-mediated intense chronic inflammation and effects of therapeutic drugs.

To identify molecules that are responsible for IgE-mediated chronic inflammation, we investigated the gene expression profiles of antigen-administered ears in TNP-IgE Tg mice by Affymetrix GeneChip analysis (Santa Clara, CA). The thickness of TNP_{11}-OVA-administered ears 24 hours after antigen challenge (just before the third-phase ear swelling) has the same degree as that of OVA-administered (control)

Table 1. Genes listed according to the magnitude of the difference between TNP_{11}-OVA and OVA in average difference, which refers to the fold value

	Average difference			
Genbank accession no.	TNP_{11}-OVA	OVA	Fold	Description
NM_010101	44.8	18.1	2.48	Sphingosine-1-phosphate receptor 3 (S1pr3)
L07379	24.8	13.0	1.91	Growth hormone–releasing hormone receptor (GHRHR)
NM_013596	45.7	24.4	1.87	Melanocortin 5 receptor (Mc5r)
NM_009219	38.3	24.2	1.58	Somatostatin receptor 4 (Sstr4)
NM_010340	87.7	55.8	1.57	G protein–coupled receptor 50 (Gpr50)
NM_008559	33.1	21.2	1.56	Melanocortin 1 receptor (Mc1r)
NM_010934	47.6	33.1	1.44	Neuropeptide Y receptor Y1 (Npy1r)
NM_015738	47.4	34.3	1.38	Galanin receptor 3 (Galr3)
AF363723	48.7	38.7	1.26	Frizzled 2 (Fzd2)

Abbreviation: TNP_{11}-OVA, 2,4,6-trinitrophenol-conjugated ovalbumin.
Four mice were used in each group for GeneChip analysis. The right and left ears were pooled in each group (TNP_{11}-OVA-injected ears or OVA-injected ears) and homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA) for total RNA preparation, as mentioned in the manufacturer's manuals. We used the GeneChip Murine Genome U74 Set arrays, which contain 36,900 mouse genes from Affymetrix (Santa Clara, CA). RNA transcription to complementary DNA, biotinylation to complementary RNA, fragmentation, hybridization to the chip, and chip analysis using the HP Gene Array Scanner were performed according to the manufacturer's manual.

Abbreviations: α-MSH, α-melanocyte-stimulating hormone; AD, atopic dermatitis; GPCR, G protein–coupled receptors; MC-R, melanocortin receptor; NDP-α-MSH, [Nle^4, D-Phe^7]-α-MSH; TNP-IgE Tg mice, 2,4,6-trinitrophenol-specific IgE transgenic mice; TNP_{11}-OVA, TNP-conjugated ovalbumin

ears (Sato *et al.*, 2003). The histological inflammatory change was not observed in both ears; however, the TNP_{11}-OVA-administered ears 24 hours after antigen challenge should change the expression of some genes, which may be responsible for inducing the third-phase inflammation. It is practicable to compare these gene expression profiles between the TNP_{11}-OVA-administered and control ears within 24 hours after antigen challenge without the influence of inflammatory cells. We compared these gene expression profiles and found genes with different expression patterns. We selected genes that were expressed at higher levels in TNP_{11}-OVA-administered ears than in control ears. Moreover, we focused on G protein–coupled receptors (GPCR), which have homology to those of humans. After these screening steps, we finally selected nine genes (Table 1). In this list, Mc1r and Mc5r, which are melanocortin receptors (MC-Rs), are expressed in both skin and immunocytes. Mc1r, Mc3r, Mc4r, and Mc5r have a common ligand, α-melanocyte-stimulating hormone (α-MSH), which has an anti-inflammatory effect on inflammatory animal models such as contact dermatitis (Grabbe *et al.*, 1996; Brzoska *et al.*, 2008). However, the mechanism of the anti-inflammatory effect of α-MSH is not clear because MC-Rs are widely distributed to various tissues, and selective agonists for each MC-Rs do not exist yet. To investigate the involvement of Mc1r and Mc5r in IgE-mediated chronic inflammation, we assessed the effect of α-MSH and the stable analog peptide [Nle^4, D-Phe^7]-α-MSH (NDP-α-MSH), which have several times higher agonist activity for Mc1r and Mc5r than α-MSH (Haskell-Luevano *et al.*, 2001) on the third-phase ear swelling in TNP-IgE Tg mice.

α-MSH and NDP-α-MSH suppressed the antigen-induced ear swelling in TNP-IgE Tg mice (Figure 1a). In particular, NDP-α-MSH significantly suppressed the third-phase ear swelling (31%) inhibitory rate in the area under the curve (ear thickness (× 0.01 mm) × time (days 2–5)). In addition, α-MSH and prednisolone marginally suppressed the immediate-phase and third-phase ear swelling (21% and 20%, respectively,

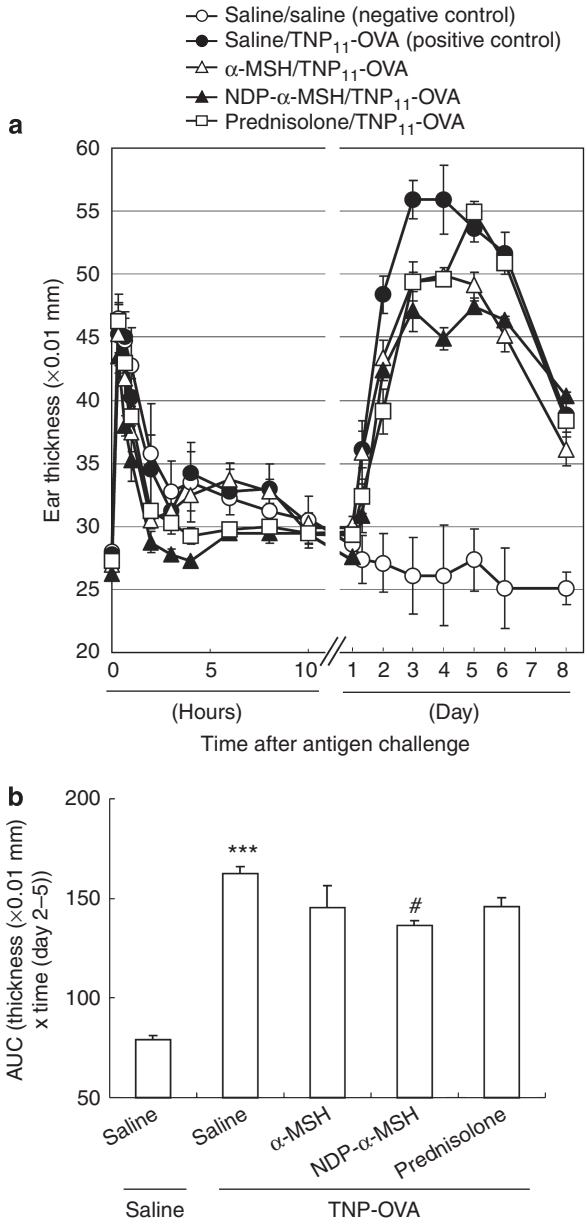

Figure 1. Inhibitory effects of α-melanocyte-stimulating hormone (α-MSH) and [Nle^4, D-Phe^7]-α-MSH (NDP-α-MSH) on the tri-phase ear swelling in 2,4,6-trinitrophenol-specific IgE transgenic (TNP-IgE Tg) mice. (**a**) The ears of TNP-IgE Tg mice were subcutaneously challenged with 1 μg TNP-conjugated ovalbumin (TNP_{11}-OVA). α-MSH, NDP-α-MSH (150 μg per body weight, each), and saline were intravenously administered 20 minutes before antigen challenge and twice a day from day 1 to 3. Prednisolone (2 mg kg^{-1}) was orally administered with the same protocol. (**b**) Area under the curve (AUC) (ear thickness (× 0.01 mm) × time (days 2–5)) on the tri-phase ear swelling. ***$P<0.001$ vs. negative control by a *t*-test. $^{\#}P<0.05$ vs. positive control by a Dunnett test. Data are expressed as the mean ± SE, $n=4$. Ear thickness was measured with a dial thickness gauge (G-1A; Ozaki, Tokyo, Japan).

Figure 1b). α-MSH and NDP-α-MSH decreased to the nanomolar range in plasma within 2 hours after intravenous administration (data not shown). Therefore, if the agonists for Mc1r and Mc5r constantly exist with high concentration in plasma, they may show stronger inhibitory effects. Artuc *et al.* (2006) showed that human mast cells generate and secrete immunoreactive amounts of α-MSH, and that the generation and secretion of α-MSH were augmented by stimulation of the mast cells with an anti-IgE antibody (Artuc *et al.*, 2006).

TNP_{11}-OVA administration in TNP-IgE Tg mice may strongly induce the generation and secretion of α-MSH from mast cells. In addition, NC/Nga mice, which develop AD-like dermatitis under conventional conditions, displayed an increase of the plasma levels of α-MSH and an increase of the expression of Mc1r and Mc5r in skin (Hiramoto *et al.*, 2009). This report in NC/Nga mice indicated a similar result to ours, which is an increase of the expression of Mc1r and Mc5r on the ears in TNP-IgE Tg mice after TNP_{11}-OVA administration.

In conclusion, we identified the nine GPCR genes that are highly expressed just before IgE-mediated chronic inflammation in TNP-IgE Tg mice. The ligands for MC1R and MC5R showed inhibitory effects on IgE-mediated chronic allergic inflammation. This result indicates that MC1R and MC5R are involved, at least partially, in the regulation of IgE-mediated chronic inflammation. We previously showed that basophils are crucially involved in the third-phase ear swelling in TNP-IgE Tg mice (Mukai *et al.*, 2005). To our knowledge, there is no available information about the expression of Mc1r and Mc5r on basophils; therefore, we assume that Mc1r and Mc5r indirectly inhibit IgE–basophil-mediated chronic inflammation. Further analysis of the mechanism of the other candidate genes could lead to the discovery of unknown mechanisms of IgE-mediated chronic allergic inflammation and a therapeutic target for treating chronic allergic inflammation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Botulinum Neurotoxin A Decreases Infiltrating Cutaneous Lymphocytes and Improves Acanthosis in the KC-Tie2 Mouse Model

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TO THE EDITOR

Psoriatic skin contains more nerve fibers (Naukkarinen *et al.*, 1989), has increased levels of sensory nerve–derived calcitonin gene-related peptide (CGRP) and substance P (SP; Chan *et al.*, 1997; Jiang *et al.*, 1998), and psoriasis disease severity can be exacerbated by stress (Griffiths and Richards, 2001), possibly by increasing cutaneous nerve numbers and SP and CGRP expression (Joachim *et al.*, 2007; Remrod *et al.*, 2007). The clinical observation that psoriasis undergoes remission following loss of innervation, nerve function, or nervous system injury (Dewing, 1971; Perlman, 1972; Farber *et al.*, 1990; Stratigos *et al.*, 1998; Joseph *et al.*, 2005) further supports a role for the nervous system in psoriasis pathogenesis; however, the

Abbreviations: BoNT-A, botulinum neurotoxin A; CGRP, calcitonin gene-related peptide ; DC, dendritic cell; PASI, psoriasis area and severity index; SP, substance P